The Influence of External Concentration on the Position of the Equilibrium attained in the Intake of Salts by Plant Cells.

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Introduction.

The intake and translocation of dissolved substances in the living plant present one of the outstanding problems of the physiology of nutrition, and one which has for a long time attracted the attention of plant physiologists.

We know the metallic or ash constituents of the plant are taken in by the root in the form of salts, and eventually find their way, in some form or another, to every part of the organism. What is the mechanism of their intake and of their translocation from cell to cell? Can the movement be explained as a simple diffusion phenomenon, or are adsorption phenomena concerned, or chemical combinations, or is the process still more complex? The work recorded in this paper forms a first instalment of an attempted analysis of these phenomena of the intake and movement of salts in the living plant.

The experimental work which has so far been performed bearing directly on our problem falls mainly into two well-defined groups. In one group the unit of experiment has been the whole living plant, and the methods employed have been those of pot-culture and particularly water-culture. Although this method of attack resulted in the discovery of the fundamental principles of plant nutrition, yet the results obtained by the walter-culture method as usually employed, although furnishing data in regard to the relationship between the constitution of a solution external to the root and the resultant growth, do not afford quantitative data as to the intake of salts. In the absence of these quantitative data general laws for the relations of electrolytes to living tissue have consequently not been formulated.

In the second group of experiments isolated cells or organs or pieces of cell-tissue form the unit of experiment. In these cases the experimental difficulties in obtaining quantitative data are considerably less than when the whole plant is dealt with, and such data are consequently accumulating. The work that forms the subject of this paper belongs to this second group, and in the experiments here recorded the parts of the plant with which we have dealt are storage organs. While it is true that the general tendency has

been for the physiology of nutrition of plants to become more and more a biochemical study of organs, or a biochemical study of the cell, rather than a study of the mode of living of the plant, yet it is perfectly obvious that it is neither possible nor desirable always to use the whole plant as the experimental object, and so long as the conditions of experiment are borne in mind, experiments on isolated cells or tissues should yield results of value which supplement those obtained by experiments with the whole living plant.

In this paper we deal with the absorption by plant tissue of salts presented singly to the tissue, and especially with the position of the equilibrium attained in this intake of salt, and the influence of the concentration of salt both on the rate of absorption and on the position of the equilibrium. Although a number of different salts were employed, the influence of the nature of the salt on the rate of its absorption is only touched upon briefly here; this question forms the subject of another paper.

Method.

The essentials of the method used are as follows. A number of discs o tissue of uniform dimensions are immersed in the experimental liquid and the change in electrical conductivity of the latter measured. In this way an approximate value is obtained of the change in ionic concentration of the external solution. The experimental tissues employed were those of potato and carrot, but, for reasons which will be explained later, carrot was chiefly used.

In order to prepare the discs, cylinders of tissue were obtained by means of a cork-borer of the necessary diameter, and the cylinders cut into discs of the required thickness by means of a hand microtome. They were then washed in distilled water thoroughly, or in tap-water followed by distilled water, and finally, lightly dried between blotting-paper before being used for an experiment.

In each experiment 100 c.c. of solution were employed, in which were immersed 40 discs of a diameter of 1·8 cm. and a thickness of 1 mm. The experiments were all performed in triplicate, which gives sufficient accuracy, as the regularity of the results obtained indicates. In all series in which the results were compared, the whole of the discs used in the series were mixed together and the sets of 40 discs for each individual sample were then taken from the general stock. It has been shown previously (13) that by this means the error arising from inherent differences in different samples of tissue may be considerably reduced. Comparisons are only made between

experiments carried out contemporaneously on discs cut at the same time and thoroughly mixed.

As it seems almost certain that the rates of absorption and exosmosis must be influenced by temperature, the experiments were all carried out in a thermostat at 20° C., and the conductivity measurements were made at the same temperature by means of Kohlrausch's method. A dipping electrode was used, which was placed direct into the experimental solutions.

A difficulty arises in such work owing to the formation of diffusion gradients between the body of the solution and the absorbing surface. The difficulty is to be overcome by breaking down the diffusion gradients over the absorbing surface. This can be effected by shaking the bottles, and accordingly this was done in our experiments. The shaker was of the usual trolley pattern and was worked by an electric motor.

Experimental Results.

(a) *Potassium, Sodium, and Calcium Chlorides (Carrot).*—The results obtained with carrot immersed in solutions of these salts in a number of concentrations ranging from N/5000 to N/10 are shown in Tables I to III. In each case a control in which the tissue was immersed in distilled water was subjected to the same conditions as the experimental solutions. It will be observed that in all cases, except those of the most dilute solutions used, the conductivity of the external solutions progressively decreases, and in the case of the weakest solutions of the various salts the rise in conductivity is less than in the case of distilled water. The results are shown graphically in fig. 1, where the initial conductivities are taken as zero. We may assume with propriety that the decrease in conductivity represents approximately the difference between the absorption of the salt by the tissue and exosmosis from the tissue. Hence the ordinates between the curves for distilled water and the salt solution can be assumed as approximately proportional to the actual amount of salt ions absorbed, or that at any rate they represent minimum values for absorption.*

* Possible causes making for a fall of conductivity in the external salt solutions *not* due to absorption and which would therefore make the values obtained greater than the true numbers for absorption :—

(1) Reactions between the exudate and the external solutions by which non-ionised molecules are produced. The possibility of such reactions appears to be ruled out as a serious source of error when we have regard to the dilution of some of the solutions and the magnitude of the decreases with higher concentrations, and further when the results that were obtained when the discs were subsequently returned to distilled water are considered.

(2) The action of sugars and other non-ionised substances in the exudate in reducing

Table I.—Carrot in Potassium Chloride of different Concentrations.

Time in hours.	Change in electrical conductivity of external solution.				
	Distilled water.	N/5000.	N/500.	N/50.	N/10.
0·5			− 3	−167	− 610
6·0	+ 80	+ 58	− 48	−372	− 970
24·0	+145	+ 92	−196	−892	−1600
52·0	+196	+137	−223	−992	−1850

Table II.—Carrot in Sodium Chloride of different Concentrations.

Time in hours.	Change in electrical conductivity of external solution.				
	Distilled water.	N/5000.	N/500.	N/50.	N/10.
3·0	+36	+30	+ 18	− 113	− 560
34·0	+87	+57	−124	− 476	−1070
41·5	+66	+19	−287	− 885	−1580
48·0	+58	+ 2	−340	−1020	−1720

conductivity of the external solutions. The quantity of such substances which diffuses out of the cells is quite negligible in this regard.

(3) A decrease in exosmosis due to the action of the salt on the tissue. Even if the extreme and unlikely assumption is made that exosmosis is reduced to nothing in all cases, the results with carrot tissue would not in the main be affected. The evidence available, however, from work on balanced solutions and the antagonistic action of ions, points in the other direction towards an increase in the rate of exosmosis under the action of single salts. [See, for example, Stiles and Jörgensen (11).]

Possible causes rendering the values for the fall in conductivity of the external salt solutions, as compared with distilled water, minimum values for absorption :—

(1) An increased exosmosis due to the action of the salts on the tissues.

(2) An independent absorption of ions. It is quite clear that by the conductivity method we shall only be measuring the approximate absorption of the least absorbed ion of a salt. When one ion of a salt enters the tissue in excess of the other, its place must be taken in the external solution by some other ion, either H, or OH (Pantanelli), or an iron escaping from the tissue (Meurer and Nathansohn). Its excess absorption will therefore not be measured. The extent to which one ion may be absorbed in excess of the other is seen as the result of the work of Nathansohn (8), Meurer (3), and Pantanelli (9). Moreover, owing to differences in mobility of different ions, and changes in the degree of ionisation resulting from replacement of one ion by another, the conductivity can only give an approximate value of the absorption.

Table III.—Carrot in Calcium Chloride of different Concentrations.

Time in hours.	Change in electrical conductivity of external solution.				
	Distilled water.	N/5000.	N/500.	N/50.	N/10.
0·5			+ 3	− 71	−343
14·5	+64	+35	− 53	−145	−457
20·5	+86	+53	− 57	−125	−370
36·25	+60	+17	−105	−181	−503
42·5	+54	+ 8	−116	−195	−470

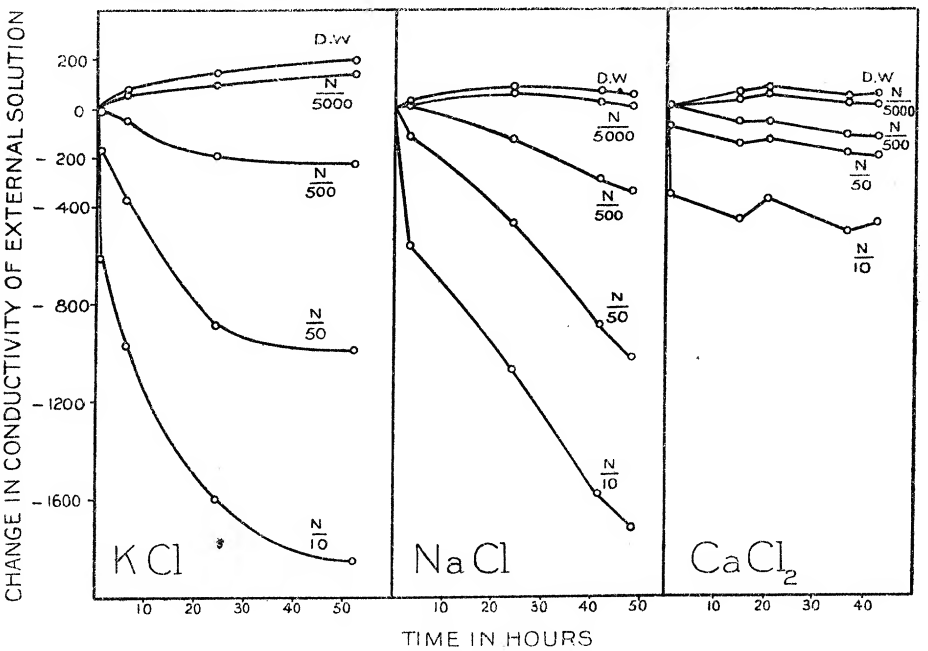

Fig. 1.—Changes in concentration of the external solution when carrot tissue is immersed in various chlorides of different concentrations.

We may note, then, first, that in all cases a decided absorption of salt takes place, and secondly, that the amounts of absorption increase along with increasing concentrations of salt in the exterior solutions. Thirdly, the rate of absorption is actually very slow at the temperatures used. In the case, for example, of the stronger concentrations of sodium and potassium chloride (N/50 and N/10) absorption is obviously not complete at the end of 48 hours. It is to be borne in mind that the discs are only 1 mm. thick. We shall return to this point later, in dealing with earlier work. Lastly, it is to be

noted that the curves for the absorption from the calcium chloride solutions show that the uptake of calcium chloride is only about a quarter of that of sodium or potassium chloride from solutions of the same equivalent concentrations. The metal ion is, of course, less concentrated in the case of calcium chloride for solutions of equal normality, but this is insufficient to account for the difference in amount of absorption.

Table IV.—Absorption of Sodium Chloride by Potato Discs as Measured by Changes in Conductivity of External Solution.

Hours.	Observed.				Calculated putting distilled water = 0.			
	D. H_2O.	N/500.	N/50.	N/10.	D. H_2O.	N/500.	N/50.	N/10.
1·15	+ 69	+ 48	− 53	−550	0	− 21	−122	− 619
14·00	+306	+277	+215	−330	0	− 29	− 91	− 636
19·40	+452	+393	+300	−343	0	− 59	−152	− 795
38·10	+681	+620	+348	−367	0	− 61	−333	−1048
47·16	+746	+614	+298	−720	0	−132	−448	−1466
62·00	+666	+557	+287	−730	0	−109	−479	−1396
86·00	+656	+498	+207	−647	0	−158	−449	−1303

Discs washed 2½ hours, running tap water and five changes of distilled water.

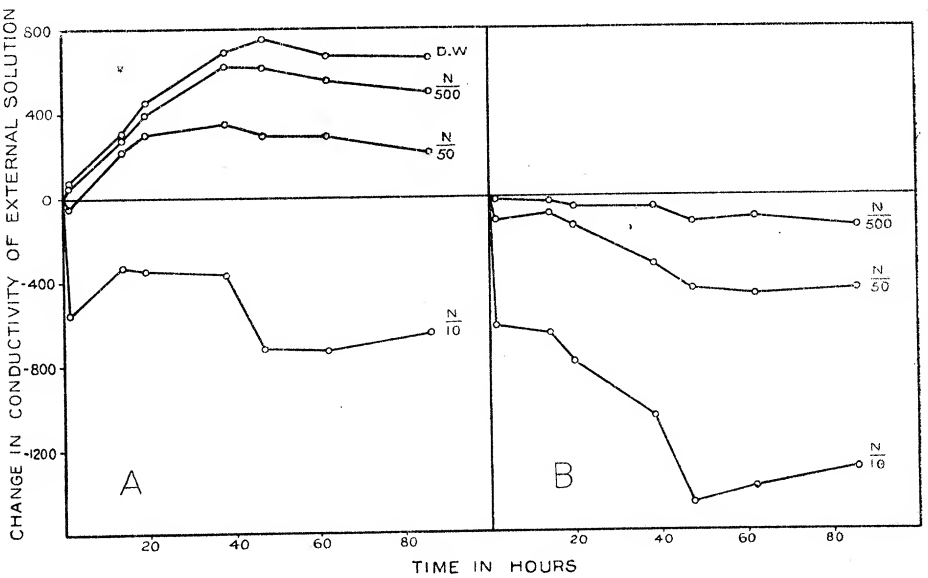

Fig. 2.—Absorption of sodium chloride by potato discs. A, changes in electrical conductivity actually measured; B, changes in conductivity relative to the change in the case of distilled water.

(b) *Sodium Chloride* (*Potato*).—The results obtained with potato tissue are complicated by the much greater exosmosis which occurs. Potato is also found to be less tolerant of the experimental conditions than carrot, and it is difficult to avoid injury and death of the discs due to secondary causes incident on the conditions of immersion. The differences between carrot and potato will be dealt with more fully in a subsequent paper, in which attention is centered on the phenomenon of exosmosis into distilled water in the case of various tissues. In the present research it has been found convenient to use carrot tissue mainly, and only a few experiments were conducted with potato.

The results of a series of experiments with sodium chloride are given in Table IV and in fig. 2. When the results are considered in the same way as those obtained with carrot tissue, that is, taking the initial conductivities as zero in all cases, and the curves representing the exosmosis into distilled water as a base line, the same conclusions appear. The amount of absorption increases with increasing concentrations of salt, and the rate of absorption is relatively slow.

(c) *Copper Sulphate* (*Carrot*).—We are here dealing with a highly toxic substance, and the results obtained (Table V and fig. 3) are of a different nature from those just described. The effect of the salt in all concentrations used is to kill the tissue by the end of the experiment. The action of the copper sulphate in all concentrations is greatly to increase the amount and rate of exosmosis from the beginning of the experiment onwards, the rate of exosmosis increasing in parallel with increasing concentrations of copper.* Exosmosis exceeds absorption in all cases. If, as we conclude, the action of the copper is very rapidly to destroy the organisation of the living cells and to render freely diffusible the full amount of contained electrolytes, this result is in accordance with expectation, as the internal concentration of salts in the tissue is equivalent to a conductivity of about 15,000, while the conductivy of the strongest solution of copper sulphate used amounts to only about 3200. The curves in fig. 3 also show that the increasing initial rate of exosmosis with increasing concentrations of copper sulphate is faster than the increasing rate of absorption of the copper sulphate. The final condition reached corresponds to an approximately equal distribution of all electrolytes (both tissue electrolyte and copper sulphate) throughout the whole system, *i.e.*, bathing liquid and tissue. This type of equilibrium condition is characteristic of dead tissue in contrast to living tissue, and is dealt with more fully in an ensuing section.

* The type of exosmosis curve obtained in the action of a toxic substance upon tissue is fully dealt with in an earlier paper. See Stiles and Jörgensen (12).

The results obtained with potato are similar. Curves showing the action of copper sulphate on this tissue as well as on the roots of living bean plants are given in an earlier paper by Stiles and Jörgensen (11). They are similar to those given here for carrot.

Table V.—Carrot in Copper Sulphate of Various Concentrations.

Time in hours.	Increase in electrical conductivity.				
	Distilled water.	N/5000.	N/1000.	N/500.	N/50.
1·5	+ 26	+ 37	+ 61	+ 81	+102
18·0	+ 75	+ 66	+ 473	+ 674	+810
43·5	+121	+997	+1139	+1394	+833

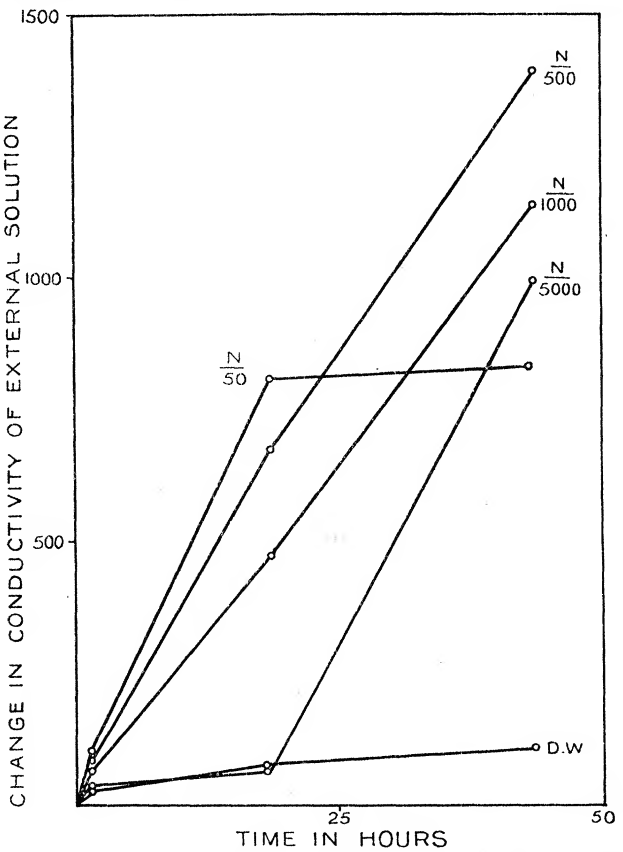

Fig. 3.—Changes in concentration of the external solution when carrot tissue is immersed in copper sulphate solutions of various concentrations.

(d) *Aluminium Sulphate* (*Carrot*).—With aluminium sulphate we are dealing with a substance which is not toxic, but which gives unusual results appearing at first sight similar to those obtained with toxic substances such as copper sulphate. In view of these results (Table VI and fig. 4), it is necessary to emphasize the marked absence of injurious action on the part of this salt even in comparison with such relatively harmless substances as potassium and sodium chlorides. If we take the more delicate potato tissue as a standard, it is very noticeable how perfectly healthy the discs remain in solutions of aluminium sulphate of all the strengths here used, in comparison with similar discs in distilled water, or in solutions of sodium or potassium chloride.

With aluminium sulphate an increase in conductivity occurred in all concentrations. The results, however, were irregular. The increase was less in N/5000 aluminium sulphate than in distilled water. It was greater in N/500, and greater again in N/50. The values in N/10, however, did not show a still further increase, but fell below those obtained in N/50. These irregularities do not appear to be due to experimental error, for exactly similar results were obtained on repeating the experiments. It is to be borne in mind also that all experiments were conducted in triplicate.

It is difficult to draw definite conclusions from these results until further analysis is made. For the present, our suggestion is that the aluminium ion is absorbed and its place taken by hydrogen ions or some other ion, which results in increasing the conductivity of the external solution. There are three lines of evidence which support this view. In the first place, as will be described in the second paper in this series, our experiments indicate that the sulphates of non-toxic metals such as potassium or sodium are very little absorbed in comparison with the chorides and nitrates. Since, as has been said above, our conductivity measurements only indicate the absorption of the least absorbed ion of a salt, this appears to show that the SO_4 ion is not easily absorbed. On the other hand, Meurer (3) has shown by direct analysis of the external solution that the aluminium ion is readily absorbed from aluminium sulphate solutions by carrot and other tissues. We quote his figures below.* Lastly, the exosmosis which occurs from the discs when subsequently returned to distilled water, as described in a succeeding section

* Meurer's results for the absorption of aluminium by potato are as follows (for the meaning of the expression absorption ratio, see at foot of next page) :—

375 grs. carrot in 750 c.c. of a 0·54 per cent. solution of $Al_2(SO_4)_3$.
Estimation of Al_2O_3 in 50 c.c. before experiment, 0·0800 grs.
After two days 0·0623, absorption ratio 0·62.
After four days 0·0600, absorption ratio 0·73.

clearly indicates that absoption increasing with increasing concentrations has taken place from the aluminium sulphate solutions.

Table VI.—Carrot in Aluminium Sulphate of various Concentrations.

Time.	Increase in electrical conductivity.				
	Distilled water.	N/5000.	N/500.	N/50.	N/10.
0·25				+ 93	+ 42
1·5	+ 57	+ 32	+ 112	+ 184	+ 130
12·42				+ 423	+ 345
14·5	+ 112	+ 109	+ 166	+ 405	
21·75	+ 98	+ 88	+ 159	+ 436	+ 375
38·75			+ 176	+ 590	+ 550
45	+ 110	+ 84	+ 136	+ 542	+ 520

Discs washed 28 hours, running tap water and five changes of distilled water.

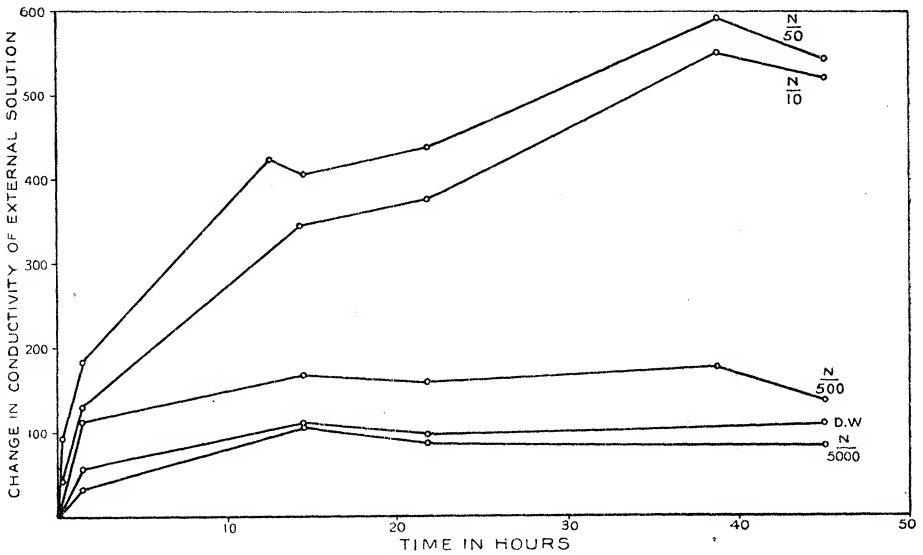

Fig. 4.—Change in electrical conductivity of external solution when carrot is immersed in aluminium sulphate of various concentrations.

375 grs. carrot in 750 c.c. of a 0·056 per cent. solution of $Al_2(SO_4)_3$.
Estimation of Al_2O_3 in 200 c.c. before experiment, 0·0337 grs.
After two days 0·0055, absorption ratio 11·33.
After four days 0·0038, absorption ratio 16·89.

Meurer's internal concentrations from which his absorption ratios are calculated are based, not on the tissue volume as in our case, but on the water content of the tissue. He concludes that the aluminium is principally absorbed by the cell-walls.

Concentration Equilibria reached in Salt Intake. "Heaping Up" of Salts in Living Tissue. Relation of External Concentration to Ratio of Internal to External Concentration at Equilibrium.

From the results described in the preceding section it is possible to obtain values for the amount of salt absorbed in any case per unit volume of tissue at any time. The values so obtained give us an expression for the "internal" concentration of the salt. Hence we can obtain the ratio of the concentration of the salt in the tissue to the concentration of the salt in the external solution.

The numbers are obtained on the assumption as before that the decrease in the conductivity of the external solution is a measure of the salt absorbed by the tissue. If the initial and final conductivities are C_1 and C_2 and X the exosmosis into distilled water, and if V is the volume of the external solution and v that of the tissue, then the ratio of the concentration of the salt in the tissue to the concentration of the salt in the external solution $= \frac{[(C_1+X)-C_2]V}{C_2 v}$. As, for the reason already discussed, C_2 is probably a higher value than actually represents the concentration of salt as compared with C_1 the number $\frac{[(C_1+X)-C_2]V}{C^2 v}$ is again a minimum value of the ratio $\frac{\text{internal concentration}}{\text{external concentration}}$. This ratio may be called the "absorption ratio."

Table VII gives the values of the absorption ratios at the end of each experiment for the different concentrations of the various salts used. It is not clear, as the graphical representations given above show, that equilibrium in absorption had been reached in all cases by this time. The curves having as ordinates the final internal concentration and as abscissæ the final external concentration, correspond to the equation $y = kc^m$, where y is the final internal concentration and c the final external concentration, and k and m are constants.

For this equation may be written in the form

$$\log y - m \log c = \log k,$$

and plotting log y against log c for our results, straight lines are obtained (figs. 5 and 6).

Although the actual values of these absorption ratios as precise measurements cannot be emphasized, the general conclusion which they indicate is remarkable.

Table VII.—Absorption Ratios in the case of Carrot and Certain Chlorides at the Approach of Equilibrium.

Initial external concentration in grm.-mols. per litre.	Relative concentrations.			Absorption ratios.
	Initial external.	Final external.	Final internal.	
KCl, 52 hours—				
N/5000	84	24	600	25·0
N/500	658	238	4,200	17·6
N/50	6,072	4,882	11,900	2·4
N/10	28,250	26,200	20,500	0·78
NaCl, 48 hours—				
N/5000	68	12	560	46·7
N/500	548	148	4,000	27·0
N/50	5,073	3,990	10,800	3·5
[N/50 (dead tissue)	5,073	5,060	4,450	0·88]
N/10	23,330	21,550	17,800	0·83
$CaCl_2$, 42·5 hours—				
N/5000	76	30	460	15·3
N/500	590	420	1,170	2·8
N/50	5,180	4,930	2,500	0·51
N/10	22,650	22,120	5,300	0·24

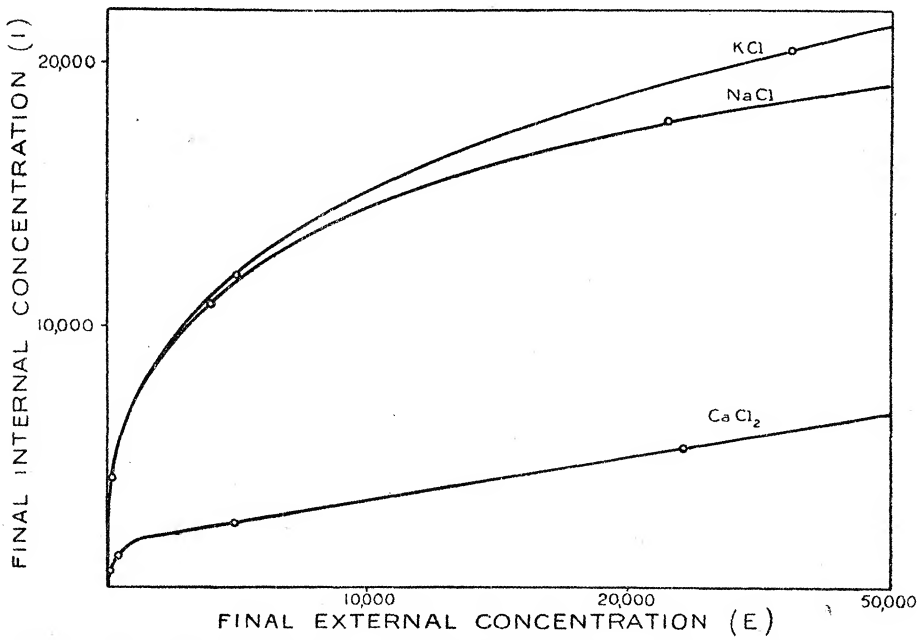

Fig. 5.—The relation between final external and final internal concentration in the case of carrot tissue immersed in certain chlorides.

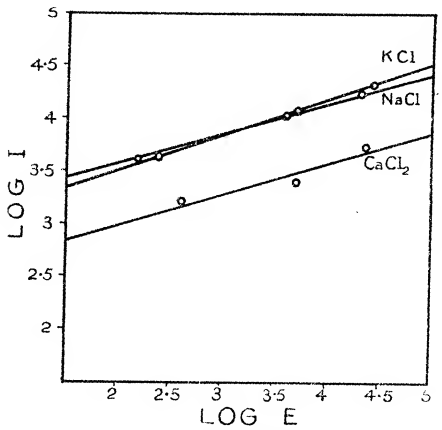

FIG. 6.—The relation between final external and final internal concentration in the case of carrot tissue immersed in certain chlorides. E is final external concentration and I final internal concentration.

It appears, in the first place, that the equilibrium reached in the absorption of a salt by living tissue depends upon the external concentration of the salt absorbed. While the *absolute amount* of absorption increases with increasing concentration of salt in the external solution as far as our experiments go, the amount relative to the external concentration nevertheless decreases rapidly from the lowest concentrations upwards.

Secondly, when the external concentrations of salt used are low, the absorption ratios become greater than unity, and with very dilute solutions probably rise to extraordinarily high values. For example, in the case of N/500 NaCl, the concentration of this salt inside the tissue after its absorption was 27 times as great as it was outside at the end of the experiment. Moreover, as we have already emphasized, this value is a minimum value for the equilibrium absorption ratio. A "heaping up" of salts is directly observed. With dead tissue, on the other hand, the equilibrium condition reached in absorption from similar solutions is one of approximately equal distribution of salt between tissues and bathing fluid.

As the concentration of the external solution is increased, the heaping up of the salt inside the tissue at equilibrium is reduced. In the highest concentrations used, *i.e.*, N/10, the degree of absorption required to produce nearly equal distribution of the salt throughout the tissue and the bathing fluid such as is characteristic in the case of dead tissue, is not reached. The absorption ratio at equilibrium is less than unity. With calcium chloride N/10, for example, where the absorption curve shows that equilibrium had been practically attained by the end of the experiment, the concentration then inside the tissue was still only about one quarter of that outside.

The calculated absorption ratios show very clearly that the degree of absorption depends to a large extent on the nature of the ions absorbed. Calcium ions are apparently far less absorbed than those of potassium or sodium.

Experiments in which at the equilibrium point reached in the intake of salts by living tissues of various kinds the absorption ratio is less than unity, that is, the final internal concentration is smaller than the external, have been previously recorded. From our results it appears that the failure to observe absorption ratios greater than unity has been due to the fact that the concentrations of the solutions used have been too high.* The results of previous workers are discussed in the following section.

Discussion of Previous Work. The Absorption Ratios found by Nathansohn and Meurer.

In his earlier work, Nathansohn (6) showed by means of chemical analyses of the external liquid and of the expressed sap that the marine alga *Codium*, in solutions of sodium nitrate, does not take up the nitrate ion to the extent required to produce equal concentrations inside and outside the tissue. The solutions he used were, however, relatively strong, varying from 0·5 per cent. to 5 per cent, that is in the region between N/20 and normal. We may quote a few of his results.

Table VIII.—Absorption Ratios in the case of Codium immersed in Sodium Nitrate. (Data from Nathansohn.)

External concentration.	Time.	Absorption ratio.
per cent.	days.	
0·5	4	0·56
0·5	10	0·68
0·5	2	0·58
1·0	5	0·44
1·0	5	0·49
3·8	2	0·43
4·8	2	0·41

Nathansohn remarked, however, that in sea-water, where the concentration of nitrate is low, the nitrate ion is often found heaped up inside the tissue, so that its concentration is greater inside than outside.

* Nevertheless, it has often been observed that the concentration of a substance or ion inside the cell could be greater than its concentration in the bathing fluid. See *e.g.*, Moore and Roaf (4, 5) and Bayliss (1) for animal cells, Wodehouse (15) for plant cells (*Valonia*).

Later work by the same author, conducted on similar lines with slices of *Dahlia* tuber (7), and of beetroot and *Helianthus* tuber (8), show that with a variety of salts used in strong concentration the tissue never takes up the quantity of salt necessary for equality of concentration in the tissue and in the external liquid. This research contains further certain interesting indications that the absorption ratio varies in the manner shown by our experiments as described above. In a series of comparable experiments with *Dahlia* slices the following figures were obtained :—

Table IX.—Absorption Ratios with Dahlia. (Data from Nathansohn.)

Salt.	External concentration.	Time.	Absorption ratio (kation).
	per cent.	days.	
NH_4NO_3	1·5	4	0·32
	0·5	4	0·53
	1·0	2	0·63
	0·5	1	0·98
$NaNO_3$	1·0	6	0·51
	0·5	5	0·94
	1·0	4	0·59
	0·5	4	1·05

These results of Nathansohn's, although the author himself did not draw the conclusion, clearly show that the absorption ratio depends upon the external concentration in the manner indicated by our experiments, namely, that there is a rapid rise in the ratio as the external concentration of the absorbed salt is increased.

Nathansohn's work was later extended by Meurer (3), who used discs of beetroot and of carrot 3 mm. in thickness, immersed in a variety of solutions, The intake of salt, or rather of ions, was measured by the change in concentration of the external solution as determined by direct chemical analysis. Meurer's results confirmed those of Nathansohn in regard to the fact that equality of concentration was never reached. We may quote Meurer's figures obtained with carrot for comparison with those recorded in this paper for the same tissues. His results with beet are parallel.

It will be observed that in all cases the individual ions of the salt have not been absorbed to the extent required for equal distribution, even after four days, the absorption ratios being for the most part less than 0·5.

As in our experiments, Meurer also compared living and dead tissue. His results, quoted below, are the same as ours. He found that in the case of dead tissue the absorption ratio for both ions was approximately unity (actually slightly less). The failure to reach a condition of equal salt

Table X.—Ratio of Ion Concentration Inside Tissue to that Outside after Intake of Salt. (Data from Meurer.)

Salt.	Duration of experiment in days.	Concentration of external solution.	Absorption ratio.	
			Kation.	Anion.
K_2SO_4	4	N/17·5	0·402	—
KNO_3	4	N/20	0·524	0·570
$NaNO_3$	4	N/17	—	0·340
KCl	2	N/15	0·374	0·287
	4	N/15	0·548	0·386
NaCl	2	N/12	0·411	0·258
	4	N/12	0·489	0·307
$CaCl_2$	2	N/14	0·270	0·229
	4	N/14	0·282	0·270
	2	N/70	0·745	0·552
	4	N/70	0·846	0·852

distribution between strong solution and tissue appears as a property of living tissue in contrast to dead tissue, just as does the heaping up of salt absorbed from weak solutions.

Table XI.—Absorption of Magnesium Chloride by Living and Dead Tissue of Carrot. (Data from Meurer.)

Concentration of external solution initially.	State of tissue.	Absorption ratios.			
		After two days.		After four days.	
		Kation.	Anion.	Kation.	Anion.
N/24	Living	0·327	0·336	0·286	0·377
N/22	Dead	0·958	0·950	0·953	0·953
N/95	Living	0·563	0·774	0·577	0·895
N/105	Dead	—	0·950	—	0·869

When we compare Meurer's results with our own, it is to be observed that just as in the case of Nathansohn's work, the figures show distinctly that there is a greater absorption relative to the concentration with decreasing concentration of the external solution. Thus, with decrease in concentration from N/24 to N/95 in the case of magnesium chloride, the concentration of magnesium in the tissue relative to that outside rises, with an immersion time of 4 days in each case, from 0·336 to 0·774. Similarly, with calcium chloride, decreasing the concentration from N/14 to N/70 increases the absorption ratio for calcium from 0·282 to 0·846 after the same period of immersion.

Meurer's results, however, show no case of the heaping up of salt inside which takes place with further dilution. This is not surprising, as the concentrations he used all lie between N/10 and N/25, with the exception of one experiment with calcium chloride at N/70 and one with magnesium chloride at N/95. Our own experiments show that with calcium chloride a much greater dilution has to be reached in order to obtain heaping up than is required with potassium or sodium chlorides.

Influence of Thickness of Tissue Slices on Absorption Ratio.

The absorption ratios observed by Meurer for sodium and potassium chloride are less than those obtained by us, though the time of immersion was twice as long. This quantitative difference may possibly be due in part to the lower temperature at which Meurer worked (5° C.) as compared with that in our experiments (20° C.), but the chief reason is undoubtedly to be found in the thickness of the discs used by him. He worked with slices of tissue 3 mm. thick, while in our experiments the thickness of the discs was only 1 mm. Ruhland (10) has already emphasized the importance of the thickness of the tissue slices used in experimental work dealing with the absorption of salts by tissue from solutions. He compared the absorption by equal weights of 3 mm. and 1 mm. discs of beet in 0·4 per cent. calcium chloride and of carrot in 1 per cent. ammonium nitrate. With neither size of disc did the absorption ratio exceed unity, which agrees with our results for such concentrations, but within the time-limits of his experiments it is seen that the 1-mm. discs absorbed considerably more than the 3-mm. discs. The following Table summarises his results. After allowing for the thickness of the discs in Meurer's experiments, they fall wonderfully into line with our own.

It is clear that the relation between surface and volume of tissue in experiments dealing with the absorption of salts from solutions needs further examination, but as far as the relation between the absorption ratio and the concentration of the external solution goes, the solution of this question should not essentially modify the results described in the present paper.

Table XII.—Influence of the Thickness of the Tissue on the Absorption of Salt by Plant Tissue. (Data from Ruhland.)

Carrot seven days in 1 per cent. ammonium nitrate.

Thickness of tissue.	Absorption ratio
3 mm.	0·5276
1 mm.	0·8342

Beet in 0·4 per cent. calcium chloride.

Thickness of tissue.	Kation.		Anion.	
	After two days.	After four days.	After two days.	After four days.
3 mm.	0·2582	0·3266	0·0354	0·0486
1 mm.	0·3421	0·5616	0·0522	0·0826

Exosmosis into Distilled Water after Immersion in Salt Solutions.

At the end of the experiments already recorded, the solutions were removed, the discs washed for a few minutes in distilled water, and then immersed in 100 c.c. of distilled water. Table XIII will serve as an example of the course of the exosmosis which results.

Table XIII.—Exosmosis into Distilled Water from Discs previously Immersed in various Concentrations of Sodium Chloride. (Rinsed quickly, once with tap water and once with distilled water.)

Time in hours.	Increase in electrical conductivity when previous solution was—				
	Distilled water.	N/5000.	N/500.	N/50.	N/10.
0·08	22	22	26	42	106
0·25	28	27	34	56	163
18·00	64	64	87	203	664
23·0	47	46	71	198	647
41·0	47	48	59	178	580
64·0	55	54	57	154	

The results show in all cases that the greater the concentration of salt in which the tissue is immersed the greater the exosmosis from the tissue on subsequent immersion in distilled water. Also, the amount of exosmosis does not increase to a constant and remain stationary, but after reaching a maximum value it slowly decreases as if electrolytes had re-entered the tissue. This phenomenon will be dealt with in a later communication.

In general, the results in regard to this exosmosis into distilled water corroborate the conclusions previously drawn as to the influence of concentration on the absorption ratio, for a similar relation is found after exosmosis into distilled water between external concentration and internal concentration, as after intake of salt.

Discussion.

From the experiments recorded in this paper, we may conclude that all the salts, or one or other of their constituent ions, readily enter cells of potato and carrot in all the concentrations employed. These two tissues show markedly different properties, exosmosis of electrolytes taking place in potato to such an extent as to mask the absorption when the electrical conductivity method is used, whereas in carrot this exosmosis is apparently negligible. For this reason, carrot is a much more suitable object for studying absorption of salts by the method employed than potato. Differences in regard to the water relations of these two tissues have been previously recorded (13), but it remains to be determined how far these different properties of the two tissues in regard to water absorption and salt exosmosis are correlated.

The substances employed fall broadly into two classes: those where the absorption does not produce any obvious injurious effect in the concentrations used, as in the case of sodium and potassium and calcium chlorides, and those where toxic action results as with copper sulphate. This division is, no doubt, arbitrary, for it is reasonable to suppose that these two classes are connected by a whole series of substances intermediate in their toxic action.

When toxic action takes place, this is accompanied by exosmosis in both potato and carrot, the initial rate of exosmosis being greater the greater the concentration of the external solution, as has previously been recorded (11).

In the case of carrot tissue immersed in solutions of potassium, sodium, and calcium chlorides, it is possible to follow the course of absorption by the electrical conductivity method. It may be said, in general, that the absorption is more rapid at first, especially for the first hour or two, and then gradually slows down until after 40 or 50 hours or more a condition of equilibrium is approached. The rapid absorption during the first minutes is particularly noticeable with the higher strengths of solutions (see the curves for N/10 solutions in fig. 1). In some recent work, Fitting (2) has concluded that the permeability of the epidermal cells of the leaf of *Rhæo discolor* as measured by the rate of entrance of potassium nitrate diminishes with time, or, in other words, that the rate of absorption of the salt decreases with time. His method of measurement of salt intake is based on the rate of deplasmolysis of the cells in solutions of the salt in question, so that only strong concentrations of the salt were employed. In a communication published while this paper was being written, Troendle (14) has recorded the results of some experiments on the absorption by bean roots and epidermal cells of *Acer platanoides* and *Salix babylonica* of salts in strong (hypertonic) solutions, the plasmolytic method being employed also here. Troendle's results are similar

to Fitting's, but he states them more precisely. His conclusion is that the rate of absorption of the salt remains unaltered for the first few minutes (ten in the case of sodium and potassium chlorides), after which it falls off with time according to a logarithmic relation where the amount absorbed is proportional to the logarithm of the time. Troendle, on the basis of these results, puts forward the hypothesis that salt irritates the protoplasm which responds by transporting salt to its interior. This produces changes in the protoplasm which are of the nature of fatigue, and which increase with time according to the Weber-Fechner law.

That the absorption of salt should take place in proportion to the logarithm of the time seems to us a totally inadequate ground for putting forward such a definite theory of salt intake. Moreover, it is not at all clear to us that Troendle's conclusions as to the course of salt intake are justified. As far as we can follow the plasmolytic method of measuring salt absorption as described by Troendle, it would appear that any possible effect of the external concentration of the salt in influencing the rate of salt absorption is neglected, and each one of his curves appears, as far as we can judge from the data presented, to be constructed from numbers obtained with a whole range of external concentrations. The results we record in this paper show that under the conditions of our experiment the external concentration has a great influence on the rate of salt intake, and it ought certainly not to be assumed that external concentration is without influence on the rate of salt absorption although, of course, we do not know without experiment whether this is the case or not with hypertonic solutions and plasmolysed cells.

Considering now the influence of concentration on the absorption of salts, an examination of the figures in Tables I–III, and the curves in fig. 1, show that the initial rate of absorption is approximately proportional to the concentration of the external solution. This relation is, however, not long maintained owing to the proportionately greater accumulation of salt in the tissue in absorption from dilute solutions. From weak external solutions the salts not only freely enter the tissues but are accumulated there, so that the internal concentration is very much higher than the external. As the external concentrations are increased this heaping up of salt in the tissue at equilibrium is proportionately less until at a certain concentration no more than equal distribution inside and outside the tissue results, while in concentrations higher than this, concentration inside the tissue at equilibrium is less than that outside. Our results with these higher concentrations agree with those of Nathansohn and Meurer.

These facts show that the absorption of salts by the tissue used cannot be explained simply by diffusion. The results suggest that combinations

take place between the salts used and some constituent or constituents of the living cells, so that either definite chemical compounds are produced, or adsorption compounds are formed.

The results obtained in regard to the influence of concentration on the absorption ratio conform to the adsorption equation. Obviously our results could be interpreted on the view that the intake of salts by the cell is simply an adsorption process, as advocated by Moore and his collaborators (4, 5), but it would also be possible to explain them on the basis of the formation of non-diffusible substances inside the cell in conjunction with changes in permeability of the cell which might result owing to the presence of the salt. Into a consideration of these questions we do not propose to enter further here. We may, however, point out that the behaviour of the treated tissues when removed from salt solution and put into distilled water suggests that the processes concerned in the intake of salts are to some extent reversible. In so transferring the treated tissues to distilled water, exosmosis occurs varying in amount according to the concentration of the solution previously used, and when the assumption is made that the exosmosis is mainly one of salt previously absorbed, it is found that the ratio of internal concentration to external concentration bears the same sort of relation to final external concentration as the absorption ratios. For any external concentration the ratio of internal to external concentration is now higher than that observed in the case of absorption ratios. These facts again make us hesitate to draw definite conclusions from the apparent conformity of the absorption results to the adsorption equation.

The influence of composition of the salt on its absorption will be dealt with in a further paper.

Summary.

1. The course of intake of salts by carrot and potato tissue has been followed by measuring the changes in conductivity of the solution of salt presented to the tissue. Concentrations of each salt were employed, varying from N/10 to N/5000.

2. In the case of copper sulphate, exosmosis exceeds absorption in all concentrations of copper sulphate. This is characteristic of toxic substances. The initial rate of exosmosis increases with increase of concentration of the toxic solution.

3. The exosmosis from carrot into distilled water is slight, while that from potato is considerable. For this reason carrot is a much more suitable subject for following absorption by the conductivity method than potato, where the absorption of salt is masked by the exosmosis of electrolytes from the tissue.

4. Carrot tissue absorbs potassium, sodium, and calcium chlorides in all concentrations examined. In the case of each salt, absorption is at first approximately proportional to the external concentration, but this relation is not continued with time, as the absorption progresses towards an equilibrium condition in which the ratio of internal to external concentration is not constant, but varies with the concentration. Similar results are obtained with potato.

5. The ratio of final internal to final external concentration has been called the absorption ratio. With low external concentrations it is many times unity, but with increasing concentration it diminishes, reaching with higher strengths of solutions a value considerably less than unity.

6. The relation between the final internal concentrations and the final external concentrations is given by the equation $y = kc^m$, where y is the final internal concentration and c the final external concentration. This is the adsorption equation, but the data presented are regarded as inadequate in themselves to justify the conclusion that absorption of salts by the cell is an adsorption process, and no proposals are put forward as to the mechanism of salt intake by the cell.

7. The results obtained are correlated with those of other workers on salt intake by plant tissue, especially those of Nathansohn, Meurer, and Ruhland in regard to salt intake as measured by direct chemical analysis, and those of Fitting and Troendle dealing with the absorption of salts from hypertonic solutions as studied by the plasmolytic method.

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On the Four Visible Ingredients in Banded Bituminous Coal: Studies in the Composition of Coal, No. 1.

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[Plates 11 and 12.]

Even after a century of investigation coal remains a complex mass of which the component parts can neither be handled nor separately identified. Many authors have recognised a variety of plant remains in coal, and the specific identification of these organisms and tissues has made good progress; but such work is truly palæontological, and the points of interest in it are the organisms and not the coal mass of which they form a part.

From another point of view coal is a rock, but, unlike most rocks, the nature and orientation of its component parts are scarcely known. One of the most distinguished of living geologists once said to me that he would like to have available about microscopic sections of coal rationalised data comparable with those already obtained by petrologists about thin rock sections. The present paper is a contribution in that direction. It is an attempt to present systematically certain observations made incidentally in